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ORIGINAL ARTICLE

Design, synthesis and evaluation of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N*-phenylacetamide derivatives as a new class of falcipain-2 inhibitors

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Abstract The cysteine protease, falcipain-2 is an important drug target in human malaria parasite *Plasmodium falciparum*. A new series of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N*-phenylacetamide derivatives **5(a–t)** were designed as per pharmacophoric requirements of falcipain-2 inhibitors using ligand-based approach. The target compounds were synthesized from the key intermediate, 2-(1,4-Diazepan-1-yl)-*N*-phenylacetamide, by coupling it with appropriate carboxylic acids using carbodiimide chemistry. Structural features of target compounds were characterized by spectral data (^{1}H NMR, and mass) and elemental analyses. The purity of the final compounds was confirmed by HPLC. The compounds were tested for their *in vitro* falcipain-2 inhibitor activity on recombinant falcipain-2 enzyme. Five compounds **5b**, **5g**, **5h**, **5j**, **5k** showed good inhibitory activity (> 60%), against falcipain-2 at 10 μM concentration, and fifteen compounds showed weak to moderate inhibitor activity. Compound **5g**, the most potent compound from this series showed 72% inhibition at 10 μM concentrations.

1. Introduction

The increasing ratio of malaria in terms of morbidity and mortality in humans turns it into a major public health problem in tropical and subtropical countries. World Health Organization Malaria report 2012 indicates that in 2010, there were 219 million malaria cases leading to approximately 660,000 malaria deaths, mostly among African children. Out of reported 90% malaria casualties in Africa alone the majority were children

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under five (Vangapandu et al., 2007). Globally, 80% of malaria deaths occur in just 14 African countries. Together, the Democratic Republic of the Congo and Nigeria account for over 40% of the estimated total of malaria death worldwide (World Malaria Report, 2012). Rapid spread of resistance is becoming most apparent in *Plasmodium falciparum* species toward first-line treatment, chloroquine and sulfadoxine-pyrimethamine (Marfurt et al., 2010). To enhance the efficacy and delayed onset of resistance, the WHO began recommending for use of Artemisinin based combination therapies (ACTs) since 2005 (Rogerson and Menendez, 2006). ACTs have potential clinical efficacy, paradoxically the history of antimalarial chemotherapy predicts that it is a matter of time before parasitic resistance emerges and spreads (Ekland and Fidock, 2008). Nevertheless, safe and cost effective novel chemical classes of antimalarial agents are urgently needed to treat malaria (Guerin et al., 2002).

Among various potential new targets, the cysteine protease falcipain-2 (FP-2) of *P. falciparum* is the most intensely studied enzyme, and its structural and functional data suggest that it is an attractive target for therapeutic intervention (Pandey et al., 2005; Wang et al., 2006). FP-2 is a principal cysteine protease that plays a major role in parasite food assimilation by its ability to degrade hemoglobin. Many *in vitro* studies have affirmed that inhibitors of falcipain-2 can inhibit the parasite hemoglobin hydrolysis and prevent the growth of culture parasites (Shenai et al., 2003; Lee et al., 2003; Domínguez et al., 1997; Huang et al., 2002). Some of them were also effective against murine malaria *in vivo* (Domínguez et al., 2005; Rosenthal et al., 1993; Sajid and McKerrow, 2002). However, the literature indicates that most of the reported FP-2 inhibitors are mainly derived from peptide analogues (Choi et al., 2013; Mallik et al., 2012; Schulz et al., 2007; Schirmeister and Kaeppler, 2003; Shenai et al., 2003; Lee et al., 2003), which have expressed nanomolar IC_{50} values, due to the formation of covalent interactions between the electrophilic groups, such as aldehydes, nitriles, vinyl sulfones and epoxides with thiolate of the catalytic cysteine amino acid. In addition, most of the existing standard ligands for falcipain-2, and cystine protease inhibitors such as Leupeptin (*N*-acetyl-L-leucyl-L-leucyl-L-argininal) (Moldoveanu et al., 2004), and E-64 (*N*-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide) (Moldoveanu et al., 2004; Varughese et al., 1989) possess chiral center(s), (Fig. 1) which increases the synthetic cost of these drugs.

This discussion clearly stresses the requirements of non-peptidic inhibitors that would bind non-covalently to the target enzyme in order to reduce toxicity and cost while retaining the potential for high activity and selectivity. The present study demonstrates the design, synthesis and *in vitro* activity of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N*-phenylacetamides as falcipain-2 inhibitors. Compounds **5(a–t)** were synthesized through the route outlined in Scheme 1. Furthermore, docking studies were performed for the active compounds to predict their relative binding affinities and binding modes in the active site of the falcipain-2 enzyme. Molecular docking studies of most active analogues (**5g**, **5h**, **5j**, **and 5k**) revealed that they interacted with Gln 36, Gln 83, Asn 173 and Hip 174 residues of 3BPF protein, via hydrogen bonding. Moreover, *in silico* pharmacokinetics and toxicity (ADMET) parameters for the selected analogues were estimated using online tool admetSAR and QikProp module of Schrodinger.

2. Materials and methods

2.1. General

Melting points (m.p.) were determined in open capillary tubes and on a Buchi 530 melting point apparatus and were uncorrected. Thin layer chromatography (TLC) was performed to monitor progress of the reaction and assess purity of the compounds; spots were detected by their absorption under UV light. ^{1}H NMR, spectra were recorded with Bruker DPX operating at 400 MHz in $CDCl_3$ or DMSO-d_6 solvent, with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown as δ values (ppm); the J values are expressed in Hertz (Hz). Signals are represented as s (singlet), d (doublet), t (triplet), q (quintet) or m (multiplet). Mass spectra (ESI) of most of the compounds exhibited molecular ion as $(M+1)^+$/$(M+Na)^+$. Purity of final compounds **5(a–t)** was evaluated on a Waters™ LC/MS system equipped with a photodiode array detector using an XBridge C18 5 µm 4.6 mm × 150 mm column. The methods used were of three types. Method A: 15% MeOH in H_2O (1 mL/min, isocratic), Method B: 25% MeOH in H_2O (1 mL/min, isocratic), and Method C: 20% CH_3CN (0.05% TFA) in H_2O (1 mL/min, isocratic). Elemental analysis was performed on a PE-2400 elemental analyzer; the C, H and N analysis was repeated twice. The chemicals were purchased commercially from Aldrich, Fluka, and Spectrochem.

2.2. Synthesis of 2-chloro-N-phenylacetamide (2)

In a round bottom flask (500 mL), compound **1** (1 g, 10.7 mmol) and triethylamine (2.9 mL, 21.4 mmol) were suspended in anhydrous DCM (200 mL) and cooled to 0 °C. To this chloroacetylchloride (1.7 mL, 21.4 mmol) was added. The reaction mixture was then stirred at room temperature

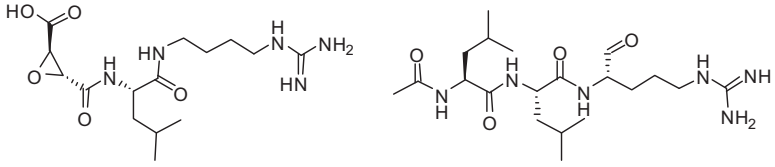
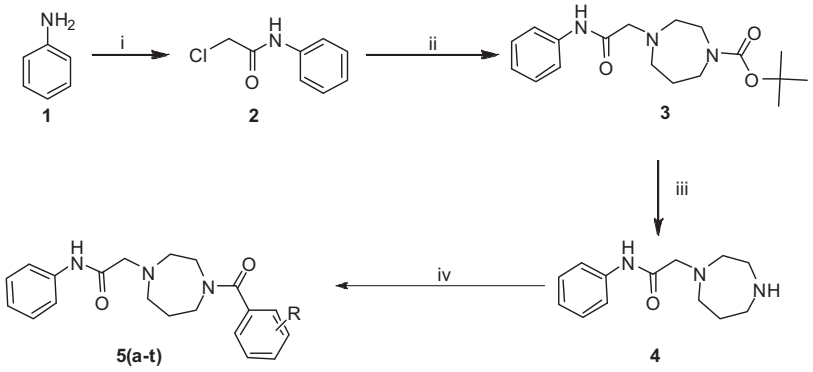


Figure 1 Existing standard ligands for falcipain-2 inhibition.



Scheme 1 Synthetic route for target compounds **5(a–t)**. Reagents and conditions: (i): Chloroacetylchloride, TEA, DCM, 0 °C-RT, 20 min; (ii): *N*-Boc-homopiperazine, K_2CO_3, CH_3CN, 100 °C, 5 h.; (iii): TFA/DCM, 0 °C-RT, 6 h; (iv): Various carboxylic acids, EDC·HCl, HOBt/DIPEA-DCM, 0 °C-RT, 6 h.

for 20 min; quenched with saturated sodium hydrogen carbonate (10 ml) and washed with water. The organic layer was separated and dried over sodium sulfate. The organic layer was evaporated under reduced pressure, and the crude reaction mixture was purified by column chromatography using chloroform and methanol as a mobile phase to obtain a pure compound **2** as a white solid. Yields 66%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (s, 1H), 7.47 (d, J = 8.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.10 (t, J = 8.0 Hz, 1H), 4.12 (s, 2H).

*2.3. Synthesis of tert-butyl 4-(2-oxo-2-(phenylamino)ethyl)-1,4-diazepane-1-carboxylate (**3**)*

To a solution of compound **2** (1.0 g, 5.8 mmol), K_2CO_3 (4.0 g, 29.4 mmol), in anhydrous acetonitrile (10 ml) was stirred for 10 min at 100 °C. To the above solution *N*-Boc-homopiperazine (1.4 ml, 7.0 mmol) was added and refluxed for 5 h. Completion of the reaction was monitored by TLC. Acetonitrile was removed under vacuum, and the reaction crude was diluted with ethyl acetate. The organic layer was then washed with water, dried over anhydrous sodium sulfate and evaporated under vacuum to get a crude mixture which was purified by column chromatography using chloroform and methanol as a mobile phase to obtain a pure compound **3** as a brown solid. Yields 68%, ^{1}H NMR ($CDCl_3$) δ: 9.16 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.27 (t, J = 8.0 Hz, 2H), 7.05 (t, J = 8.0 Hz, 1H), 3.52 (s, 2H), 3.45 (m, 2H), 3.25 (m, 2H), 2.79 (m, 4H), 1.84 (m, 2H), 1.40 (s, 9H).

*2.4. Synthesis of 2-(1,4-diazepan-1-yl)-N-phenylacetamide (**4**)*

To a solution of **3** (1.0 g, 3.0 mmol) in anhydrous DCM (20 mL) in a round bottom flask at 0 °C, trifluoroacetic acid (1.1 mL, 15.0 mmol) was added. The reaction mixture was stirred at room temperature for 6 h. Upon completion of the reaction, solvent was removed under vacuum. To the residue, saturated solution of sodium bicarbonate was added, and extracted with ethyl acetate. Ethyl acetate portion was evaporated to obtain the free amine as off white solid. Yield: 70%, ^{1}H NMR ($CDCl_3$) δ: 9.14 (s, 1H), 7.56 (dd, J = 8.0 Hz, 1 Hz, 2H), 7.25 (m, 2H), 7.04 (m, 2H), 3.28 (s, 2H), 3.12 (m, 4H), 2.89 (m, 2H), 2.81 (m, 2H), 1.92 (m, 2H).

*2.5. General procedure for the synthesis of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide derivatives **5(a–t)***

To an appropriate carboxylic acid (1 g), DIPEA (2.4 equiv), EDC·HCl (1.5 equiv), and HOBt (0.8 equiv) in anhydrous DCM (10 ml) were added and the reaction was stirred for 5 min at 0 °C. To this reaction mixture the compound **4** (1.1 equiv) was added. The reaction was stirred for 6 h at room temperature and then solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate, and the organic phase was washed with 5% aqueous sodium bicarbonate solution (twice) and saturated brine solution (once). The organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure, and the crude product was purified by column chromatography.

*2.5.1. 2-(4-Benzoyl-1,4-diazepan-1-yl)-N-phenylacetamide (**5a**)*

Semi solid, purity 99% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.10 (s, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.0 Hz, 5H), 7.33 (q, J = 7.8 Hz, 2H), 7.12 (t, J = 7.0 Hz, 1H), 3.84 (m, 2H), 3.55 (m, 2H), 3.34 (s, 1H), 3.26 (s, 1H), 3.02 (m, 1H), 2.88 (m, 1H), 2.81 (m, 2H), 2.04 (m, 1H), 1.85 (m, 1H); MS (ESI): m/z 338.18 $(M+1)^+$; Anal. calcd for $C_{20}H_{23}N_3O_2$ C, 71.19; H, 6.87; N, 12.45; found C, 71.21; H, 6.81; N, 12.41.

*2.5.2. 2-(4-(3-Fluorobenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5b**)*

White solid, purity 97% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.09 (s, 1H), 7.61 (m, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.46 (m, 2H), 7.34 (m, 3H), 7.12 (m, 2H), 3.83 (m, 2H), 3.57 (m, 2H), 3.44 (s, 1H), 3.16 (s, 1H), 3.12 (m, 1H), 3.01 (m, 3H), 2.12 (m, 1H), 1.92 (m, 1H); MS (ESI): m/z 356.19 $(M+1)^+$; Anal. calcd for $C_{20}H_{22}FN_3O_2$ C, 67.59; H, 6.24; N, 11.82 found C, 67.61; H, 6.21; N, 11.81.

*2.5.3. 2-(4-(2-Fluorobenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5c**)*

Brown solid, purity 96% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.08 (s, 1H), 7.61 (dd, J = 8.5, 1.0 Hz, 1H), 7.55

(dd, J = 8.5, 1.0 Hz, 1H), 7.37 (m, 4H), 7.21 (m, 1H), 7.12 (m, 2H), 3.87 (m, 2H), 3.48 (m, 2H), 3.34 (s, 1H), 3.26 (s, 1H), 3.01 (m, 1H), 2.88 (dd, J = 6.2, 5.1 Hz, 1H), 2.81 (dd, J = 10.8, 6.7 Hz, 2H), 2.04 (m, 1H), 1.86 (m, 1H); MS (ESI): m/z 356.19 $(M+1)^+$; Anal. calcd for $C_{20}H_{22}FN_3O_2$ C, 67.59; H, 6.24; N, 11.82; found C, 67.61; H, 6.21; N, 11.81.

*2.5.4. 2-(4-(3-Chlorobenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5d**)*

Semi solid, purity 95% (method A); ^{1}H NMR ($CDCl_3$) δ: 8.99 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.40 (m, 2H), 7.33 (m, 4H), 7.12 (t, J = 7.2 Hz, 1H), 3.83 (m, 2H), 3.57 (m, 1H), 3.53 (t, J = 6.2 Hz, 1H) 3.37 (s, 1H), 3.28 (s, 1H), 3.04 (m, 1H), 2.85 (m, 3H), 2.06 (m, 1H), 1.89 (m, 1H); MS (ESI): m/z 371.21 $(M)^+$ and 372.23 $(M+1)^+$; Anal. calcd for $C_{20}H_{22}ClN_3O_2$ C, 64.60; H, 5.96; N, 11.30; found C, 64.62; H, 5.97; N, 11.31.

*2.5.5. 2-(4-(4-Chlorobenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5e**)*

Semi solid, purity 95% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.16 (s, 1H), 7.61 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 7.4 Hz, 1H), 7.36 (m, 6H), 7.12 (t, J = 7.4 Hz, 1H), 3.83 (m, 2H), 3.53 (m, 2H), 3.38 (s, 1H), 3.28 (s, 1H), 3.05 (m, 1H), 2.85 (m, 3H), 2.06 (m, 1H), 1.88 (m, 1H); MS (ESI): m/z 371.21 $(M)^+$ and 372.23 $(M+1)^+$; Anal. calcd for $C_{20}H_{22}ClN_3O_2$ C, 64.60; H, 5.96; N, 11.30; found C, 64.62; H, 5.97; N, 11.31.

*2.5.6. 2-(4-(2-Chlorobenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5f**)*

Semi solid, purity 98% (method C); ^{1}H NMR ($CDCl_3$) δ: 9.06 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 7.6 Hz, 2H), 7.48 (m, 5H), 7.21 (t, J = 7.6 Hz, 1H), 3.80 (m, 2H), 3.57 (m, 2H), 3.48 (s, 1H), 3.38 (s, 1H), 3.15 (m, 1H), 3.05 (m, 3H), 2.36 (m, 1H), 1.78 (m, 1H); MS (ESI): m/z 371.21 $(M)^+$ and 372.23 $(M+1)^+$; Anal. calcd for $C_{20}H_{22}ClN_3O_2$ C, 64.60; H, 5.96; N, 11.30; found C, 64.62; H, 5.97; N, 11.31.

*2.5.7. N-Phenyl-2-(4-(3-(trifluoromethyl)benzoyl)-1,4-diazepan-1-yl) acetamide (**5g**)*

White solid, purity 96% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.04 (s, 1H), 7.70 (d, J = 5.7 Hz, 2H), 7.61 (d, J = 7.8 Hz, 2H), 7.54 (dd, J = 13.9, 7.8 Hz, 2H), 7.34 (q, J = 7.8 Hz, 2H), 7.13 (m, 1H), 3.86 (m, 2H), 3.56 (m, 1H), 3.51 (t, J = 6.4 Hz, 1H) 3.38 (s, 1H), 3.29 (s, 1H), 3.06 (m, 1H), 2.87 (m, 2H), 2.09 (m, 2H), 1.90 (m, 1H); MS (ESI): m/z 406.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{22}F_3N_3O_2$ C, 62.21; H, 5.47; N, 10.36; found C, 62.22; H, 5.45; N, 10.35.

*2.5.8. N-Phenyl-2-(4-(4-(trifluoromethyl)benzoyl)-1,4-diazepan-1-yl)acetamide (**5h**)*

Yellow solid, purity 95% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.04 (s, 1H), 7.69 (t, J = 8.1 Hz, 2H), 7.61 (d, J = 7.6 Hz, 1H), 7.54 (t, J = 8.5 Hz, 3H), 7.34 (q, J = 8.1 Hz, 2H), 7.13 (t, J = 7.6 Hz, 1H), 3.86 (m, 2H), 3.55 (t, J = 5.1 Hz, 1H), 3.50 (t, J = 6.2 Hz, 1H), 3.36 (s, 1H), 3.28 (s, 1H), 3.04 (m, 1H), 2.90 (m, 1H), 2.82 (m, 2H), 2.07 (m, 1H), 1.87 (m, 1H); MS (ESI): m/z 406.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{22}F_3N_3O_2$ C, 62.21; H, 5.47; N, 10.36; found C, 62.22; H, 5.45; N, 10.35.

*2.5.9. N-Phenyl-2-(4-(2-(trifluoromethyl)benzoyl)-1,4-diazepan-1-yl) acetamide (**5i**)*

White solid, purity 94% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.04 (s, 1H), 7.69 (m, 2H), 7.61 (d, J = 8.0 Hz, 1H), 7.54 (m, 3H), 7.34 (m, 2H), 7.13 (t, J = 8.0 Hz, 1H), 3.90 (m, 2H), 3.55 (d, J = 6.2 Hz, 1H), 3.49 (t, J = 6.2 Hz, 1H), 3.38 (s, 1H), 3.30 (s, 1H), 3.12 (m, 1H), 2.95 (m, 1H), 2.87 (m, 2H), 2.12 (m, 1H), 1.85 (m, 1H); MS (ESI): m/z 406.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{22}F_3N_3O_2$ C, 62.21; H, 5.47; N, 10.36; found C, 62.22; H, 5.45; N, 10.35.

*2.5.10. 2-(4-(2-Methoxybenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5j**)*

Semi solid, purity 96% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.16 (s, 1H), 7.63 (dd, J = 8.6, 1.0 Hz, 1H), 7.54 (m, 1H), 7.34 (m, 3H), 7.23 (m, 1H), 7.11 (m, 1H), 6.99 (m, 1H), 6.92 (dd, J = 8.3, 4.5 Hz, 1H), 3.95 (m, 1H), 3.84 (m, 1H), 3.81 (s, 3H), 3.45 (m, 1H), 3.35 (s, 2H), 3.26 (m, 1H), 2.99 (m, 1H), 2.81 (m, 4H), 2.04 (m, 1H); MS (ESI): m/z 368.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_3$ C, 68.64; H, 6.86; N, 11.44; found C, 68.65; H, 6.87; N, 11.46.

*2.5.11. 2-(4-(3-Methoxybenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5k**)*

Semi solid, purity 95% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.09 (s, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.33 (dd, J = 14.8, 7.8 Hz, 3H), 7.12 (d, J = 7.0 Hz, 1H), 6.97 (m, 3H), 3.86 (m, 1H), 3.81 (s, 3H), 3.78 (m, 1H), 3.55 (m, 2H), 3.34 (s, 1H), 3.26 (s, 1H), 3.01 (m, 1H), 2.88 (m, 1H), 2.80 (m, 2H), 2.04 (m, 1H), 1.85 (m, 1H); MS (ESI): m/z 368.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_3$ C, 68.64; H, 6.86; N, 11.44; found C, 68.65; H, 6.87; N, 11.46.

*2.5.12. 2-(4-(4-Methoxybenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5l**)*

Semi solid, purity 99% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.11 (s, 1H), 7.57 (m, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.33 (t, J = 7.3 Hz, 2H), 7.11 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 3.84 (m, 1H), 3.83 (s, 3H), 3.79 (m, 1H), 3.59 (m, 2H), 3.30 (s, 2H), 3.01 (m, 1H), 2.95 (m, 1H), 2.82 (m, 2H), 2.04 (m, 1H), 1.87 (m, 1H); MS (ESI): m/z 368.21 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_3$ C, 68.64; H, 6.86; N, 11.44; found C, 68.65; H, 6.87; N, 11.46.

*2.5.13. 2-(4-(3-Ethoxybenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5m**)*

Semi solid, purity 98% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.17 (s, 1H), 7.67 (m, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 7.3 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 6.90 (d, J = 8.4 Hz, 2H), 4.15 (q, J = 8.0 Hz, 2H), 3.85 (m, 1H), 3.78 (m, 1H), 3.61 (m, 2H), 3.40 (s, 2H), 3.19 (m, 1H), 2.90 (m, 1H), 2.83 (m, 2H), 2.04 (m, 1H), 1.87 (m, 1H), 1.32 (t, J = 8.0 Hz, 3H); MS (ESI): m/z 382.31 $(M+1)^+$; Anal. calcd for $C_{22}H_{27}N_3O_3$ C, 69.27; H, 7.13; N, 11.02; found C, 69.24; H, 7.11; N, 11.04.

*2.5.14. 2-(4-(2-Ethoxylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5n**)*

Semi solid, purity 96% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.12 (s, 1H), 7.77 (m, 2H), 7.57 (d, J = 8.0 Hz, 2H), 7.37 (t, J = 7.3 Hz, 2H), 7.23 (t, J = 7.3 Hz, 1H), 6.79

(d, J = 8.0 Hz, 2H), 4.21 (q, J = 8.0 Hz, 2H), 3.88 (m, 1H), 3.82 (m, 1H), 3.71 (m, 2H), 3.45 (s, 2H), 3.29 (m, 1H), 2.89 (m, 1H), 2.81 (m, 2H), 2.14 (m, 1H), 1.97 (m, 1H), 1.30 (t, J = 8.0 Hz, 3H); MS (ESI): m/z 382.31 $(M+1)^+$; Anal. calcd for $C_{22}H_{27}N_3O_3$ C, 69.27; H, 7.13; N, 11.02; found C, 69.24; H, 7.11; N, 11.04.

*2.5.15. 2-(4-(4-Ethoxybenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5o**)*

Semi solid, purity 95% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.14 (s, 1H), 7.62 (m, 2H), 7.51 (t, J = 8.4 Hz, 2H), 7.37 (m, 2H), 7.21 (d, J = 8.4 Hz, 1H), 6.90 (m, 2H), 4.19 (q, J = 8.1 Hz, 2H), 3.81 (m, 1H), 3.80 (m, 1H), 3.71 (m, 2H), 3.34 (s, 2H), 3.29 (m, 1H), 2.92 (m, 1H), 2.79 (m, 2H), 2.14 (m, 1H), 1.84 (m, 1H), 1.28 (t, J = 8.1 Hz, 3H); MS (ESI): m/z 382.31 $(M+1)^+$; Anal. calcd for $C_{22}H_{27}N_3O_3$ C, 69.27; H, 7.13; N, 11.02; found C, 69.24; H, 7.11; N, 11.04.

*2.5.16. 2-(4-(3-Methylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5p**)*

Semi solid, purity 98% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.10 (s, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.41 (m, 4H), 7.21 (m, 2H), 7.06 (m, J = 7.3 Hz, 1H), 3.90 (m, 2H), 3.65 (m, 2H), 3.31 (s, 1H), 3.20 (s, 1H), 3.12 (m, 1H), 2.78 (m, 3H), 2.05 (m, 1H), 2.33 (s, 3H) 1.79 (m, 1H); MS (ESI): m/z 352.23 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_2$ C, 71.77; H, 7.17; N, 11.96; found C, 71.76; H, 7.18; N, 11.95.

*2.5.17. 2-(4-(4-Methylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5q**)*

Semi solid, purity 96% (method B); ^{1}H NMR ($CDCl_3$) δ: 9.11 (s, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.32 (m, 4H), 7.21 (m, 2H), 7.11 (t, J = 7.3 Hz, 1H), 3.83 (m, 2H), 3.55 (dd, J = 14.2, 7.9 Hz, 2H), 3.34 (s, 1H), 3.26 (s, 1H), 3.02 (m, 1H), 2.83 (m, 3H), 2.05 (m, 1H), 2.38 (s, 3H) 1.85 (m, 1H); MS (ESI): m/z 352.23 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_2$ C, 71.77; H, 7.17; N, 11.96; found C, 71.76; H, 7.18; N, 11.95.

*2.5.18. 2-(4-(2-Methylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5r**)*

Semi solid, purity 97% (method A); ^{1}H NMR ($CDCl_3$) δ: 9.10 (s, 1H), 7.62 (m, 1H), 7.53 (m, 1H), 7.32 (m, 3H), 7.20 (m, 3H), 7.12 (m, 1H), 3.92 (m, 2H), 3.37 (m, 4H), 3.06 (s, 1H), 2.87 (m, 2H), 2.75 (s, 1H), 2.34 (s, 3H), 2.07 (m, 1H), 1.84 (m, 1H); MS (ESI): m/z 352.23 $(M+1)^+$; Anal. calcd for $C_{21}H_{25}N_3O_2$ C, 71.77; H, 7.17; N, 11.96; found C, 71.76; H, 7.18; N, 11.95.

*2.5.19. 2-(4-(4-Ethylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5s**)*

Semi solid, purity 96% (method C); ^{1}H NMR (DMSO-d_6) δ: 10.98 (s, 1H), 7.79 (s, 1H), 7.68 (d, J = 7.9 Hz, 2H), 7.38 (m, 2H), 7.28 (m, 3H), 7.11 (t, J = 7.9 Hz, 1H), 4.32 (m, 2H), 3.85 (m, 1H), 3.59 (m, 4H), 3.26 (s, 2H), 2.68 (q, J = 7.6 Hz, 2H), 2.58 (m, 2H), 2.48 (m, 1H), 1.25 (t, J = 7.6 Hz, 3H); MS (ESI): m/z 366.28 $(M+1)^+$; Anal. calcd for $C_{22}H_{27}N_3O_2$ C, 72.30; H, 7.45; N, 11.50; found C, 72.31; H, 7.42; N, 11.51.

*2.5.20. 2-(4-(3-Ethylbenzoyl)-1,4-diazepan-1-yl)-N-phenylacetamide (**5t**)*

Semi solid, purity 94% (method B); ^{1}H NMR (DMSO-d_6) δ: 10.78 (s, 1H), 7.80 (s, 1H), 7.66 (d, J = 7.9 Hz, 2H), 7.38 (m, 2H), 7.30 (m, 3H), 7.21 (t, J = 7.9 Hz, 1H), 4.30 (m, 2H), 3.75 (m, 1H), 3.57 (m, 4H), 3.46 (s, 2H), 2.66 (q, J = 7.6 Hz, 2H), 2.60 (m, 2H), 2.58 (m, 1H), 1.95 (t, J = 7.6 Hz, 3H); MS (ESI): m/z 366.28 $(M+1)^+$; Anal. calcd for $C_{22}H_{27}N_3O_2$ C, 72.30; H, 7.45; N, 11.50; found C, 72.31; H, 7.42; N, 11.51.

2.6. Enzyme assay

The protocol for the purification and refolding of recombinant protein falcipain-2 as described by Shenai et al. (2000) and Korde et al. (2008) was followed. In brief, a mixture of 100 mM NaOAc, 10 mM DTT, 6 μM enzyme and 10 μM of test inhibitors at pH 5.5, 10 mM of fluorogenic substrate benzyloxycarbonyl-Phe-Arg-7-amino-4-methylcoumarin hydrochloride (ZFR-AMC) was added and the release of 7-amino-4-methylcoumarin (AMC) was monitored (excitation 355 nm; emission 460 nm) over 30 min at RT using Perkin Elmer Victor 3 multi-label counter. Preliminary assays were performed to determine the percentage inhibition of the enzyme at a concentration of 10 μM based on DMSO as control represented in Table 1.

2.7. Docking studies

To understand the structural basis for the activities of the inhibitors and to support the *in vitro* activity results, we

Table 1 Falcipain-2 inhibitor activity profile of final compounds **5(a–t)**.

Compound	R	Inhibition rate[c] at 10 μm%	Compound	R	Inhibition rate at 10 μm%
5a	-H	49	**5k**	*m*-OCH_3	64
5b	*m*-F	61	**5l**	*p*-OCH_3	55
5c	*o*-F	60	**5m**	*m*-OCH_2CH_3	32
5d	*m*-Cl	47	**5n**	*o*-OCH_2CH_3	36
5e	*p*-Cl	47	**5o**	*p*-OCH_2CH_3	30
5f	*o*-Cl	36	**5p**	*m*-CH_3	49
5g	*m*-CF_3	72	**5q**	*p*-CH_3	47
5h	*p*-CF_3	68	**5r**	*o*-CH_3	48
5i	*o*-CF_3	50	**5s**	*p*-CH_2CH_3	30
5j	*o*-OCH_3	70	**5t**	*m*-CH_2CH_3	24
E-64		97.4			

[c] Data are mean of three independent experiments and the deviations are < 5% of the mean value.

studied the binding models of the top four active analogues; **5g**, **5h**, **5j** and **5k** with falcipain-2 enzyme using Glide 5.9 (Schrodinger, LLC, New York, NY, 2013) running on maestro version 9.4 installed in a machine on Intel Xenon W 3565 processor and Cent OS Linux Enterprise version 6.3 as the operating system (Friesner et al., 2004; Halgren et al., 2004). The crystal structure of falcipain-2 (PDB entry 3BPF) from *P. falciparum* was retrieved from the Protein Database Bank with a resolution of 2.9 Å. The downloaded FP-2 protein carries four chains named A, B, C, and D; complexed with epoxysuccinate E64 (Wang et al., 2000). The catalytic triad of Cys 42, Asn 173 and His 174 is located in the cleft between the two structurally distinct domains (Wang et al., 2014). Protein preparation module of Schrodinger suite was used for protein preparation. Proteins were pre-processed separately by deleting the substrate co-factor as well as the observed water molecules were removed from the coordinate set, followed by optimization of hydrogen bonds. Charge and protonation state was assigned and energy was minimized with Root Mean Square Deviation (RMSD) value of 0.3 Å using Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field (Jorgensen et al., 1996). Potential of non-polar parts of receptors was softened by scaling van der Walls radii of ligand atoms by 1.00 Å to generate the grid. Analogues and Standard Drug E-64 structures were drawn using ChemSketch and converted to 3D structure with the help of 3D optimization tool. LigPrep module was used to optimize the geometry of the drawn ligands.

3. Results and discussion

Existing falcipain-2 inhibitors, (Desai et al., 2004; Li et al., 2009) were used as a template for making a pharmacophore model for falcipain-2 inhibitors, and some of the compounds (**I**–**VIII**), which possess moderate to potent activities are represented in Fig. 2. Among the represented compounds, particular imine (**I**) and phenyl hydrazones (**IV** and **VIII**), probably inhibit the enzyme by covalent interactions.

The most common features present in the aforementioned falcipain-2 inhibitors are, an aromatic residue (monocyclic/bicyclic) which is attached to the hydrophobic moiety; commonly an aromatic residue through a hydrogen bond donor and acceptor atom(s) as linker. The distance between the aromatic residue and the hydrophobic group ranged from 9 to 14 Å. The hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) atom(s) are present as either in heterocyclic/alicyclic or open chain form. The numbers of hydrogen bond donor and acceptor atoms range from 0 to 2 and 2 to 6, respectively. The reported molecules are basic in nature due to 2° or 3° amino moiety. By considering these common features as pharmacophore for falcipain-2 inhibitors, a pharmacophore model was built as shown in Fig. 3. Among the broad range of heterocyclic templates particularly, the piperazine core is found as the most encouraging leading heterocyclic ring, present in a number of drugs and clinical candidates that address a broad spectrum of serious targets (Han et al., 2012). Therefore, initially we synthesized some derivatives based on piperazine nucleus, and tested *in vitro* against falcipain-2 enzyme; unfortunately, the insignificant activity of the compounds (unpublished observation), prompted us to focus our efforts to increase the ring size especially, on utilizing 1,4-diazepam as a core nucleus (Ettari et al., 2009; Micale et al., 2006), with the aim of improving the drug like profile of this novel class of compounds, as shown by basic structure **5(a–t)** in Scheme 1.

The least energy conformation (three minimum energy conformations for each compound) for each designed compound was generated by ACDLABS-12.0 product version 12.01/3D viewer (CHARMM parameterizations), and the pharmacophoric distances were measured from the centroid of an aromatic residue to a hydrophobic residue. The observed distances between the pharmacophoric elements of all the

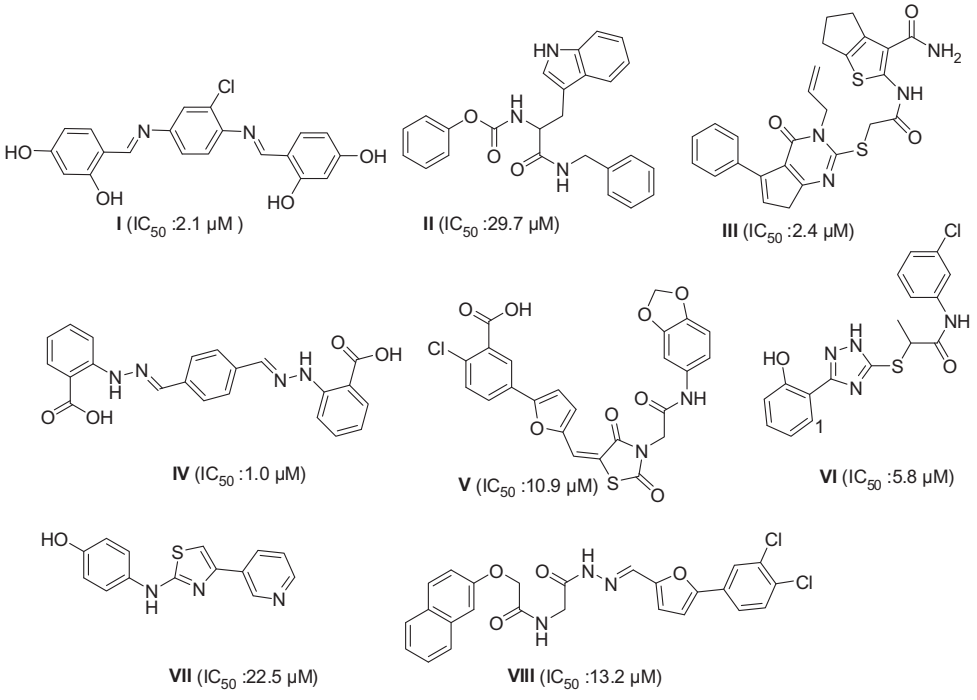


Figure 2 Structures of some reported falcipain-2 inhibitors.

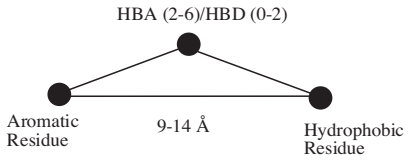


Figure 3 Pharmacophoric features for falcipain-2 inhibitors.

designed compounds are in agreement with our proposed pharmacophore model. To achieve the better pharmacokinetic profile, Lipinski's Rule of Five (Lipinski et al., 1997) was adopted for the designed molecules. Lipophilicity is an important parameter to be considered while designing ligand to manifest drug-like behavior. Thus, Log *P* values of all the designed molecules were calculated utilizing JME Molecular Editor (Courtesy of Peter Ertl, Novartis).

The target compounds were prepared as outlined in Scheme 1. First, compound **4** was synthesized in multi-gram scale from the starting material aniline in a sequence of reactions. Chloroacetyl chloride was subjected to nucleophilic substitution reaction with aniline, which afforded compound **2**. This intermediate was reacted with *N*-Boc protected homopiperazine, to obtain compound **3**. Subsequently, deprotection of the Boc group with trifluroacetic acid furnished the intermediate 2-(1,4-diazepan-1-yl)-*N*-phenylacetamide **4**. This key intermediate was coupled with appropriate carboxylic acids in the presence of coupling agents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) under nitrogen atmosphere to afford target compounds **5(a–t)**. Synthesized compounds were isolated as pure and characterized by ^{1}H NMR, mass spectroscopy, HPLC and elemental analysis. The analytical and spectral data of the compounds confirmed the structures and purities of the final compounds.

All synthesized compounds were evaluated for their *in vitro* falcipain-2 inhibitor activity. Several compounds showed significant inhibitory activity (> 60%), against falcipain-2 at 10 μM presented in Table 1, and their chemical structures and physical constants are shown in Table 2.

Retaining the common 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N* phenylacetamide framework, compounds **5a**, **5b** were screened, initially. Fortunately, these two compounds **5a** and **5b** exhibited inhibitory activity against falcipain-2 enzyme at 10 μM concentrations with inhibition values of 49% and 61%, respectively. Based on its moderate potency and synthetic feasibility, compound **5b** served as a solid starting point for the future drug discovery program. Thus, various substitutions were introduced around the scaffold based on **5b** specifically in the aromatic moiety. The effect of the fluorine group, an electron withdrawing substituent, was investigated at position 2 on the phenyl moiety (compound **5c**). This modification did not result in any improved potency, and

Table 2 Physical constants of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N*-phenylacetamide derivatives **5(a–t)**.

Compound	R	Molecular Weight	Molecular Formula	% Yield[a]	m.p. (°C)	Log *P*[b]
5a	-H	337.44	$C_{20}H_{23}N_3O_2$	62	Semi solid	1.79
5b	*m*-F	355.40	$C_{20}H_{22}FN_3O_2$	82	125–127	1.95
5c	*o*-F	355.40	$C_{20}H_{22}FN_3O_2$	68	118–120	1.95
5d	*m*-Cl	371.86	$C_{20}H_{22}ClN_3O_2$	81	Semi solid	2.35
5e	*p*-Cl	371.86	$C_{20}H_{22}ClN_3O_2$	65	Semi solid	2.35
5f	*o*-Cl	371.86	$C_{20}H_{22}ClN_3O_2$	62	Semi solid	2.35
5g	*m*-CF_3	405.41	$C_{21}H_{22}F_3N_3O_2$	68	122–124	2.71
5h	*p*-CF_3	405.41	$C_{21}H_{22}F_3N_3O_2$	61	156–158	2.71
5i	*o*-CF_3	405.41	$C_{21}H_{22}F_3N_3O_2$	61	140–142	3.22
5j	*o*-OCH_3	367.44	$C_{21}H_{25}N_3O_3$	64	Semi solid	1.66
5k	*m*-OCH_3	367.44	$C_{21}H_{25}N_3O_3$	73	Semi solid	1.66
5l	*p*-OCH_3	367.44	$C_{21}H_{25}N_3O_3$	87	Semi solid	1.66
5m	*m*-OCH_2CH_3	381.46	$C_{22}H_{27}N_3O_3$	66	Semi solid	2.0
5n	*o*-OCH_2CH_3	381.46	$C_{22}H_{27}N_3O_3$	71	Semi solid	2.0
5o	*p*-OCH_2CH_3	381.46	$C_{22}H_{27}N_3O_3$	71	Semi solid	2.0
5p	*m*-CH_3	351.44	$C_{21}H_{25}N_3O_2$	66	Semi solid	2.27
5q	p-CH_3	351.44	$C_{21}H_{25}N_3O_2$	81	Semi solid	2.27
5r	*o*-CH_3	351.44	$C_{21}H_{25}N_3O_2$	62	Semi solid	2.27
5s	*p*-CH_2CH_3	365.40	$C_{22}H_{27}N_3O_3$	70	Semi solid	2.69
5t	*m*-CH_2CH_3	365.46	$C_{22}H_{27}N_3O_2$	79	Semi solid	2.69

[a] Yields refer to isolated pure compounds.
[b] Log *P* values are calculated by using JME Molecular Editor (Courtesy of Peter Ertl, Novartis).

Table 3 Results of docking simulations for the most active molecules using Glide software.

Name of ligand	G-score	Lipophilic EvdW	H bond	XP Electro
5g	−6.405	−3.426	−1.583	−0.7663
5h	−6.380	−3.509	−1.582	−0.7637
5j	−6.404	−3.720	−2.439	−0.5968
5k	−6.128	−3.744	−2.096	−0.5546

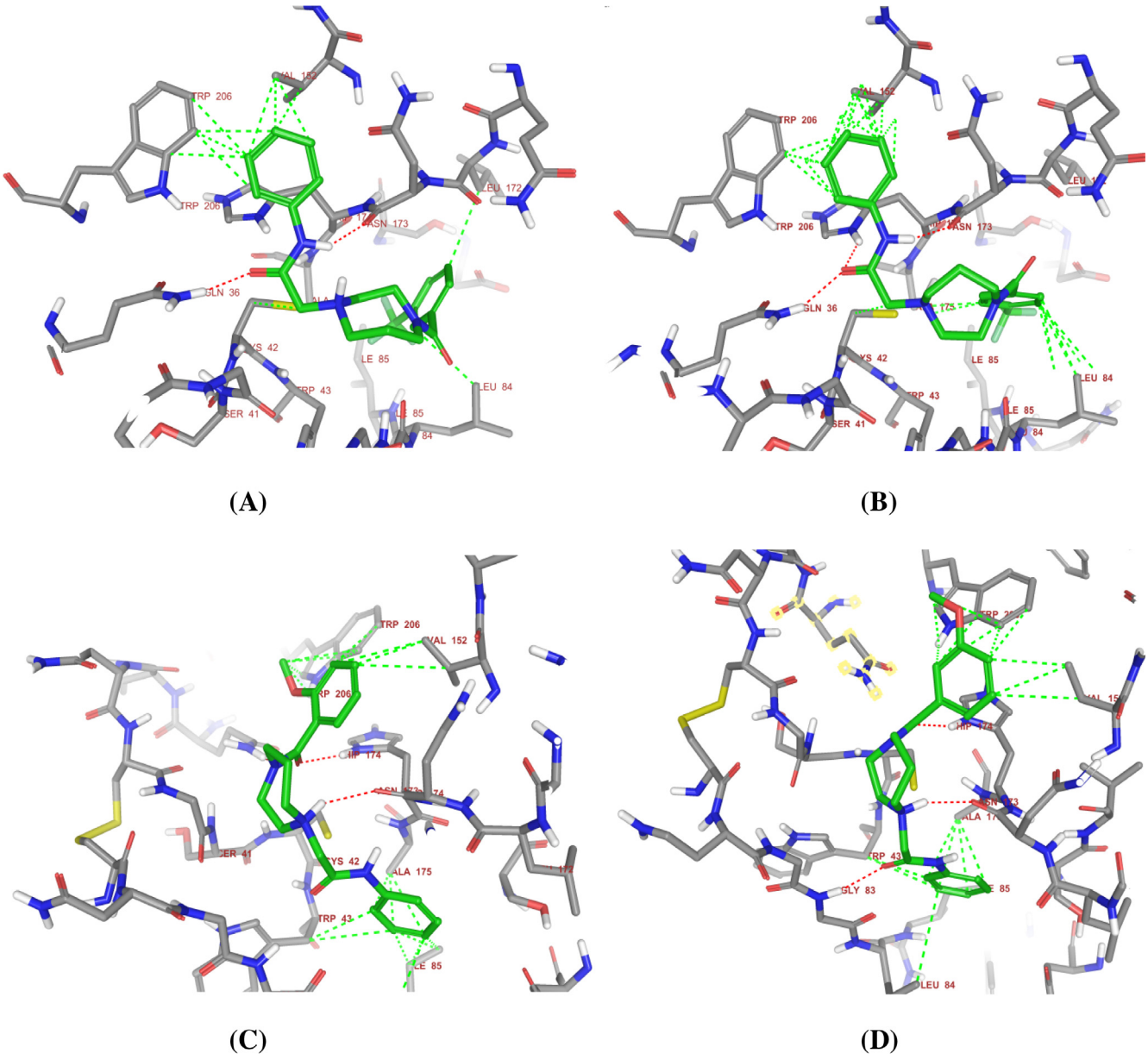


Figure 4 (A–D) Predicted binding poses of **5g**, **5h**, **5j**, **5k** respectively docked to chain A of falcipain-II protein (3BPF.pdb). The red dashed line represents the possible hydrogen bond and green dashed line represents the possible hydrophobic interaction.

compound exhibited 60% enzyme inhibition value close to the compound **5b**. Placement of another electron withdrawing substituent such as chloro in the aromatic ring, generated molecules (**5d**, **5e**, **5f**) with less potency (47% inhibition by **5d** and **5e**, and 36% by **5f**). When a strong electron withdrawing group, trifluoromethyl was introduced at the 3 position to get the compound **5g** (72% inhibition) and position 4 to get the compound **5h** (68% inhibition), both compounds showed higher potency than the hit compound **5b**. However, investigation of a trifluoromethyl group at the 2 position (compound **5i**) of the phenyl ring showed lesser potency (50% inhibition) compared to compound **5b**.

Consequently, a methoxy group, an electron releasing substituent was introduced at 2 and 3 positions of the phenyl ring resulting in compounds **5j** and **5k**, with improved inhibition potency (70% and 64%), greater than the hit compound **5b**

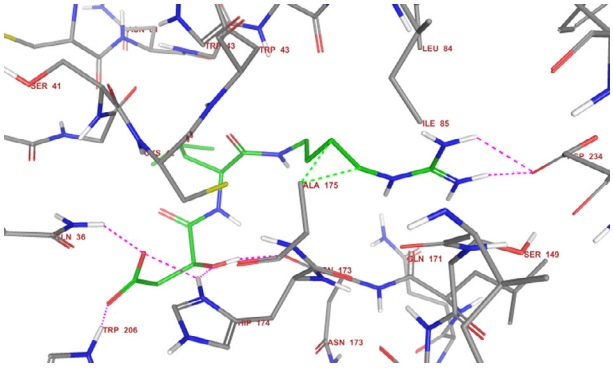


Figure 5 Best docked pose of E-64 inside falcipain-2. The pink dashed line represents the possible hydrogen bond and green dashed line represents the possible hydrophobic interaction.

from this series. Attachment of the methoxy group in the 4 position of the phenyl ring gave rise to compound **5l**, with inhibition potency (55%), which was lesser than that of **5b**. Compounds **5m**, **5n** and **5o** achieved by replacement of the methoxy group with the ethoxy group lose (32%, 36% and 30%) potency markedly. Replacement of the methoxy group in the phenyl ring with another weak electron releasing group such as methylene (**5p**, **5q** and **5r**) at 2, 3 and 4 positions and the ethylene (**5s** and **5t**) group at 3 and 4 positions leads to a decrease in the potency.

This discussion clearly indicates that the strong electron withdrawing group (trifluoromethyl) at 3 and 4 positions and a strong electron releasing group (methoxy) at 2 and 3 positions generated compounds that displayed marked inhibition as evidenced by compounds **5g**, **5h**, **5j**, and **5k** compared to the non-substituted derivatives i.e., compound (**5a**). Higher homologation of electron releasing groups, methoxy to ethoxy (**5m**, **5n**, **5o**) and methylene to ethylene (**5s**, **5t**) in the phenyl ring generated compounds with lesser inhibition.

The prepared ligands were docked with proteins using extra precision mode (XP). The best docked pose obtained from Glide was analyzed. Results of docking simulations using Glide are summarized as XP Gscore, lipophilic energy, H-bond energy and electrophoretic energy in Table 3. Comparison of interacting residues of these analogues (**5g**, **5h**, **5j**, **5k**) shows that the amino acids Ala 175, Trp 43, Ile 85, Leu 84, Cys 42, Trp 206, Val 152 and Asp173 of falcipain-II protein are most commonly involved as shown in Fig. 4(A–D). Among these, the residue Asp 173 was the hydrogen bonding common interacting residue of falcipain-II with ligands (**5g**, **5h**, **5j**, **5k**). Compound **5g** showed eight hydrophobic interactions, Ala 175, Trp 43, Ile 85, Leu 84, Leu172, Cys 42, Trp 206, Val 152; two hydrogen bonds, Gln 36 (2.08 Å), Asn 173 (2.19 Å). Compound **5h** showed nine hydrophobic interactions, Ala 175, Trp 43, Ile 85, Leu 84, Leu 172, Cys 42, Trp 206, Val 152, Ile 84; two hydrogen bonds, Asn 173 (2.25 Å), Gln 36 (2.27 Å). Compound **5j** showed seven hydrophobic interactions, Ala 175, Trp 43, Ile 85, Leu 84, Cys 42, Trp 206, Val 152; two hydrogen bonds, Asn 173 (2.34 Å), Hip 174 (2.06 Å). Compound **5k** showed nine hydrophobic interactions, Ala 175, Trp 43, Ile 85, Leu 84, Leu 172, Cys 42, Trp 206, Val 152, Val 150; three hydrogen bonds, Asn 173 (2.51 Å), Hip 174 (1.93 Å), Gln 83 (2.49 Å).

In the present study, validation of the docking was done by removing co-crystallized ligand E-64 from the active site and subjecting it again to dock into the binding pocket in the conformation found in the crystal structure. It is important to note that E-64 is a covalent inhibitor of several cysteine proteases and is potent non-selective due to the formation of covalent bond with the active site thiol (Li et al., 2009; Schiefer et al., 2013). Therefore, to validate the docking protocol, we used E-64 with an open epoxide ring as reported in the literature (Mugumbate et al., 2013). Furthermore, a set of studies were performed to compute the RMSD value between the pose of co-crystallized inhibitor present in the enzyme and its best ranked pose (Fig. 5), in the protein. As a result, the RMSD value calculated for co-crystallized ligand, E 64 was <2 Å against the target enzyme. The best scoring pose of the reference drug inside the FP-2 showed that interactions are in resemblance with the reported X-ray pose interactions (Kerr et al., 2009). These results suggested that our docking procedure, and software protocol could be relied on to predict the experimental binding mode of the designed analogues.

As discussed earlier, physicochemical properties such as molecular weight (180–500), log P_o/w (−0.4 to 5.6), of all the designed analogues follow Lipinski's Rule of Five. Furthermore, Qik–prop module of Schrodinger, and online software admetSAR (http://www.admetexp.org/predict/), were used to generate *in silico* pharmacokinetics and physicochemical parameters for compounds **5g**, **5h**, **5j** and **5k** in order to estimate their drug-likeness properties. The recommended range (Ntie-Kang et al., 2013; Cheng et al., 2012) of some physicochemical and pharmacokinetic parameters, which plays an important role to both pre-clinical research and clinical development, such as: log S (−6.0 to 0.5), log BB (−3.0 to 1.0), % Human oral absorption ($\leqslant$25% is poor and $\geqslant$80% is high) was predicted (Table 4). In addition, AMES toxicity, acute oral toxicity and carcinogenicity were also predicted and represented as qualitative terms with probability (in bracket) in Table 4. Range of Caco-2 cell permeability gives a view to select a molecule as lead compound at early stage by determining the capability to transport about across the cell membrane of the gut. The physicochemical properties and *in silico* predicted ADMET properties as mentioned in Tables 2 and 4, for selected analogues are in the acceptable range of druggable profile.

Table 4 *In-silico* predicted admet properties of selected compounds.

Properties	5g	5h	5j	5K	Range for drug likeness
Log S[d]	−4.20	−4.20	−3.04	−3.28	−6.5–0.5
Log BB[e] (probability)	0.983	0.982	0.953	0.971	–
Human oral absorption (%)[f]	97.5	97.2	95.8	96.0	$\geqslant$80% is high, $\leqslant$25% is poor
Caco-2 permeability[g]	1.103	1.103	1.255	0.935	–
AMES toxicity (probability)	Non toxic (0.707)	Non toxic (0.707)	Non toxic (0.764)	Non toxic (0.789)	–
Carcinogens (probability)	Non-carcinogen (0.8372)	Non-carcinogens (0.8372)	Non-carcinogens (0.9062)	Non-carcinogens (0.9104)	–
Acute oral toxicity[h] (probability)	III 0.6166	III 0.6166	III 0.7543	III 0.7813	–

[d] Predicted aqueous solubility in moles/liter.
[e] Predicted brain/blood partition coefficient.
[f] Percentage of human oral absorption.
[g] Predicted apparent Caco-2 cell permeability (nm/s).
[h] Category III includes compounds with LD50 values [500 mg/kg but\5000 mg].

Although, representatives of this new series did not exhibit strong falcipain-2 activities as compared to the existing non-peptidic FP-2 inhibitors such as chalcones (Li et al., 1995; Liu et al., 2001), which are biosynthetic precursors of flavonoids, thiosemicarbazones (Chiyanzu et al., 2003) and isoquinolines (Sajid and McKerrow, 2002; Batra et al., 2003). However, this work shows that indirect drug design protocol can be successfully expedited for the discovery of small molecule cysteine protease inhibitors. Overall, the discussed efforts toward the design of inhibitors of the malarial cysteine proteases clearly demonstrate the effectiveness of combining computational techniques with organic synthesis in delivering novel potential inhibitors and in providing directions for further improvements.

4. Conclusion

In summation, a series of 2-(4-(substituted benzoyl)-1,4-diazepan-1-yl)-*N*-phenylacetamide with twenty novel derivatives, using ligand based approach were synthesized and screened for falcipain-2 inhibition. The structures of the compounds were assigned on the basis of ^{1}H NMR and mass spectral data. The preliminary SARs indicate that, overall, the compounds having 3-F (**5b**), 3-CF_3 (**5g**), 4-CF_3 (**5h**), 2-OCH_3 (**5j**) and 3-OCH_3 (**5k**) substituent in the aromatic ring displayed marked inhibition. Compound **5g** is the most potent compound from this series, with lesser toxicity predicted by softwares (admetSAR and QikProp module of Schrodinger), and it can be utilized as a potential lead compound in the designing of new candidates to optimize the inhibitory potencies of this class of compounds, with potent antimalarial activity. Docking result provided the fundamental structural information to maintain the inhibitory activity, and was helpful for future inhibitor design. Moreover, to get the conclusive results, *plasmodia* study has to be done which will be the futuristic extension of the work. Thus, the present approach could be an excellent starting point for the development of novel cysteine protease inhibitors, and in general, for the development of new drugs for malaria.

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